Is the innate bio-protection power against human virus the same between males and females? A conclusion based on blood donor data of HTLV-I infection

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Human T-cell leukemia virus type I (HTLV-I) is a retrovirus that causes adult T-cell leukemia. The male-to-female transmission is stronger than the reverse, so the carrier proportion of women is greater than that of men. On the other hand, since the mother-to-child transmission route via the breast-feeding is common for baby boys and girls, it has been thought the HTLV-I proportions of boys and girls are the same until now. A question arises as to whether the 'innate protection powers against human virus' are the same between baby boys and girls. We utilize Blood donor data in 1995-1998, which were provided by Japan Red Cross Society of Oita, Japan. The data are summarized into the frequency table with respect to gender and age. The age groups are < 20, 20 < age ≤ 30, 30 < age ≤ 40, 40 < age ≤ 50, and >

50 years old. The comparison of carrier proportions of males and females under 20 years old is made with a two-sided statistical test and for the other groups one-sided tests are carried out. The preset statistical analysis shows that the carrier proportion of girls is less than that that of boys. It implies that in HTLV-I the mother-to-child transmission probability of females is less than that of males. According to the present findings, it follows that the female's innate protection power against HTLV-I is stronger than that of males, and the conclusion may become a valid proposition for general human virus.

Human T-cell Leukemia Virus Type I (HTLV-I) is a retrovirus that causes Adult T-cell Leukemia (ATL) and myelopathy/tropical paraparesis[1-4]. The carriers of this virus live on the Japanese Islands, the African Continent, in South America, the Caribbean Islands, Australia, and elsewhere[5-8]. In Japan, about one percent of the people are carriers, and fifty percents of these carriers live on Kyushu and Okinawa Islands[3,4,9]. In Japan, nationwide epidemiological studies have been made[10-12]. From epidemiological studies of HTLV-I infection, the following infection routes are established as the main routes[4,6,13,14]: (i) from infected mothers to their newborn babies via the mother's milk; (ii) from infected males (husbands) to susceptible females (their wives) through frequent sexual intercourse; and (iii) from infected females (wives) to males (husbands). Other minor transmission routes seem to be the intrauterine mother-to-child transmission and the transmission via saliva[15,16].

For detecting HTLV-I carriers, serological assays, e.g. the indirect immunofluorescence method (IF), enzyme-linked immunosorbent assay (ELISA) and gelatin particle agglutination method (PA), are usually used to diagnose HTLV-I

carriers. In Japan, IF was used for blood donor data by 1994; however a detection of HTLV-I carriers with only this method is insufficient for screening antibodies to HTLV-I-associated antigens[17,18]. In Japan, since 1995 three methods, i.e. IF, ELISA and PA, have been simultaneously employed to screen the antibody to HTLV-I more precisely. As far as these tests, the results are incomplete and the HTLV-I prevalence is underestimated with the usual serological assays[19]. It was reported that there was a HTLV-I seronegative and infected South Indian patient with chronic spastic paraparesis[20], and seronegative rat models with HTLV-I infection were also experimentally established[21-23]. Thus, testing for Tax sequences and antibodies to its gene product, i.e. the polymerase chain reaction (PCR) method is needed for screening HTLV-I carriers in blood transfusion[19,24,25]. By using the PCR method, HTLV-I provirus was detected from seronegative Chilean patients with tropical spastic paraparesis[26], and defective HTLV-I provirus was also derived from some seronegative Chilean patients with the disease[27]. In an HTLV-I endemic area in Japan, i.e. Tushima, 209 healthy adults were examined for HTLV-I provirus by PCR and anti-HTLV-I antibodies by PA, ELISA, and IF; however none of the 133 seronegative subjects, i.e. seropositive subjects were 76, reacted positively on PCR. Hence, seronegative HTLV-I carriers are considered to be rare in Japan[28].

In considering HTLV-I infection, the mother-to-child transmission probability is assumed to be the same between males and females, i.e. the innate immunity or bio-protection powers of males and females are equivalent (e.g. 5, 14, 29). Doubtless the assumption has been employed since the virus was detected. In this paper, through statistical analysis of blood donor data in Oita prefecture, Japan, we derive a conclusion that the innate bio-protection power of females against HTLV-I may be stronger than that of males.

Summary of Blood Donor Data of HTLV-I in 1995 to 1998

Table 1 shows the ‘blood donor data in 1995 to 1998’ provided by Japan Red Cross Society of Oita. The data are summarized in subgroups according to donors' age. The data are random samples from not the natural population in Oita prefecture, Japan, but the *blood donor population* which is defined below. Recently, HTLV-I carriers have chances to be screened through the blood donation and blood tests. Especially, pregnant women are tested their blood in order to prevent the mother-to-child transmission. In Japan, pregnant women infected with HTLV-I have recommended not to feed their babies on mother's milk since 1985. From this measure, carriers of infants are decreasing every year[30,31]; however blood donors in Table 1 is not affected from this medical intervention, because ages of blood donors in Japan are greater or equal to fifteen years old and the data were obtained in 1995 to 1998. Thus, AG_1 can be viewed as a population without the screening effect, i.e. a natural population. The effect of screening on AG_2 and AG_3 of females is very strong, because the majority of the reproductive population in Japan is in the age groups and HTLV-I infection is informed to pregnant women concerned. If a pregnant woman is found to be a HTLV-I carrier, her husband may also have his blood examined in most cases. From this, the screening effect on males in AG_2 and AG_3 is also strong. From 1999 the carriers detected in blood donors are informed the HTLV-I infection; however the screening effect did not affect the data in Figure 1. Hence, the carrier proportion in blood donors is decreasing every year. A population from which a part of HTLV-I carriers were screened is referred to as a *blood donor population* (Fig. 1). The data in Table 1 are regarded as random samples from the blood donor population in each year, i.e. repeated measurement data. The estimated carrier proportions in age groups are illustrated in Figures 2 to 6. In AG_1, the carrier proportions of boys are higher than those of girls in all four years. From this, the mother-to-child transmission probability of boys may be higher than that of girls. By using the HTLV-I carrier data of healthy residents of Okinawa, Japan, in periods 1968-

1970, 1981-1984, and 1996-1999, Kashiwagi et al[31] reported that there was no significant difference between boys and girls less than 20 years old. However, the data are small in considering the HTLV-I prevalence of children, i.e. 4 of 106 in boys and 5 of 90 in girls in 1968-1970; 2 of 209 in boys and 5 of 204 in girls in 1981-1984; and 1 of 522 in boys and 0 of 417 in girls in 1996-1999 are carriers. The prevalence of HTLV-I carriers is small, i.e. about 1% to 5%, so large sample data are needed to derive more precise results. As shown in Table 1, the sizes of blood donor data are large, so a more powerful test for comparing the HTLV-I carrier proportions of males and females can be made with the data. The reverse process in carrier proportions of males and females with age may be illustrated in Figures 2 to 6. Moreover, a screening effect on carrier proportions may be shown in the Figures.

Statistical analysis in carrier proportions

Let $\pi_{(M,a)}(t)$ be the proportion of males who are infected in AG_a at year t and let $\pi_{(F,a)}(t)$ be the proportion of females who are infected in AG_a at year t (t = 1995,1996,1997,1998; a = 1,2,3,4,5). In testing the differences with respect to carrier proportions between males and females in 5 age groups, the following null and alternative hypotheses are employed:

$H_{(1,0)}$: $\pi_{(M,1)}(t) = \pi_{(F,1)}(t)$; $H_{(1,1)}$: $\pi_{(M,1)}(t) \neq \pi_{(F,1)}(t)$,

$H_{(a,0)}$: $\pi_{(M,a)}(t) = \pi_{(F,a)}(t)$; $H_{(a,1)}$: $\pi_{(M,a)}(t) < \pi_{(F,a)}(t)$, a = 2,3,4,5.

For level of significance $\alpha = 0.05$, the results are shown in Table 2. According to the results, the carrier proportion of males is higher than that of females under twenty. Since any medical intervention effects may be negligible in the age group, it can be concluded that the mother-to-child transmission probability of newborn baby boys is

higher than that of girls. This is a new finding obtained in this study. It may be remarkable, because as mentioned above the mother-to-child transmission probability has been assumed the same between boys and girls. The other results in Table 2 support the usual finding that the male-to-female transmission rate is higher than the female-to-male transmission one.

Discussion

Although it has been thought that there is no difference in HTLV-I infection between boys and girls under twenty years old, from Figure 2 one may doubt whether it stands to reason. From the above statistical analysis, the HTLV-I carrier proportion of boys was significantly greater than that of girls under twenty, i.e. AG_1. The HTLV-I infection in AG_1 depends on the mother-to-child transmission by postnatal breast-feeding[16]. Through the reproductive population, the infection proportions are reversed between males and females. It follows that in the sexual transmission the female is more easily infected than the male; however the infection depends on the transmission routes. On the other hand, the present study has shown that the baby girl has a lower probability than the baby boy in mother-to-child transmission. The route of mother-to-child transmission is the same for baby boys and girls. The existence of window period of HTLV-I infection was reported[32]; however, seronegative HTLV-I carriers in Japanese healthy residents are rare[28]. In a follow-up study in Japan, none of 21 seronegative children born to seropositive mothers was seroconverted, and the HTLV-I provirus was not detected with PCR[33]. Hence, it follows that there are cases where invasions of HTLV-I through breast-feeding are completely defended without acquiring the

immunity against the virus and that the innate bio-protection power of females against HTLV-I is superior to that of males.

According to sex hormones the immunity of women may be stronger than that of men; however the present result has been derived from the mother-to-child transmission through breast-feeding and it does not depend on sex hormones. Thus, it may reach a valid conclusion that the female's innate bio-protection power against human virus is stronger than that of males. By analyzing the difference between the 'innate bio-protection systems against human virus' of males and females, a new protection measure against human virus may be developed. Experimental studies with animal models and epidemiological approaches with respect to human virus are required to explain and prove the proposition.

Methods

Let $N_{(M,a)}(t)$ and $n_{(M,a)}(t)$ be the numbers of male blood donors and HTLV-I infected males sampled from AG_a at year *t*, respectively; and let $N_{(F,a)}(t)$ and $n_{(F,a)}(t)$ be those for females in AG_a at year *t*, respectively. The maximum likelihood estimates of infection proportions are given by

$$\hat{\pi}_{(M,a)}(t) = \frac{n_{(M,a)}(t)}{N_{(M,a)}(t)},\ \hat{\pi}_{(F,a)}(t) = \frac{n_{(F,a)}(t)}{N_{(F,a)}(t)}\ \ (a = 1,2,3,4,5;\ t = 1995,1996,1997,1998).$$

From the central limit theorem, the above statistics are independent and asymptotically distributed according to normal distributions with means $\pi_{(M,a)}(t)$ and variances $\hat{\sigma}_{(M,a)}(t)^2 = \frac{\hat{\pi}_{(M,a)}(t)\left(1-\hat{\pi}_{(M,a)}(t)\right)}{N_{(M,a)}(t)}$ for males and with $\pi_{(F,a)}(t)$ and $\hat{\sigma}_{(F,a)}(t)^2 = \frac{\hat{\pi}_{(F,a)}(t)\left(1-\hat{\pi}_{(F,a)}(t)\right)}{N_{(F,a)}(t)}$ for females, respectively. The differences between carrier proportions of males and females in age groups are tested. The test statistics used are

$$Z_{(a)} = \frac{\sum_{t=1995}^{1998}\left(\hat{\pi}_{(M,a)}(t) - \hat{\pi}_{(F,a)}(t)\right)}{\sqrt{\sum_{t=1995}^{1998}\left(\hat{\sigma}_{(M,a)}(t)^2 + \hat{\sigma}_{(F,a)}(t)^2\right)}} \quad (a = 1,2,\ldots,5). \qquad (1)$$

The above statistics are asymptotically distributed according to the standard normal distribution under the null hypotheses, i.e. $H_{(a,0)}$: $\pi_{(M,a)}(t) = \pi_{(F,a)}(t)$, $a = 1,2,...,5$.

Statistics (1) are used for testing the differences with respect to carrier proportions between males and females in 5 age groups.

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Table 1. Blood Donor Data in 1995 to 1998 in Oita Prefecture, Japan

year	1995		1996	
gender	male	female	male	female
AG_1 [15,20)*	44 (3852)**	38 (5135)	31 (3914)	27 (5459)
AG_2 [20,30)	66 (9935)	69 (8860)	75 (10606)	72 (9636)
AG_3 [30,40)	125 (9759)	77 (3955)	140 (9891)	73 (4307)
AG_4 [40,50)	248 (10016)	143 (3697)	248 (10858)	136 (3965)
AG_5 [50,60)	168 (6374)	156 (3084)	199 (6620)	160 (3135)

year	1997		1998	
gender	male	female	male	female
AG_1 [15,20)	30 (3615)	24 (4781)	23 (2813)	21 (4245)
AG_2 [20,30)	79 (10708)	58 (9638)	73 (10718)	78 (10470)
AG_3 [30,40)	115 (10349)	69 (4646)	112 (10365)	46 (5117)
AG_4 [40,50)	229 (11100)	127 (4087)	194 (10530)	126 (3950)
AG_5 [50,60)	200 (7224)	140 (3449)	218 (7610)	136 (3570)

*) [*a*,*b*) implies ages from *a* to *b* years old.

**) The numbers in parentheses are numbers of donors.

Table 2. Comparison of carrier proportions of males and females in age groups

Age Group	AG_1	AG_2	AG_3	AG_4	AG_5
Z Statistic	3.879	− 0.555	− 2.080	− 8.776	− 8.488
P val.	0.000	0.289	0.0188	0.000	0.000

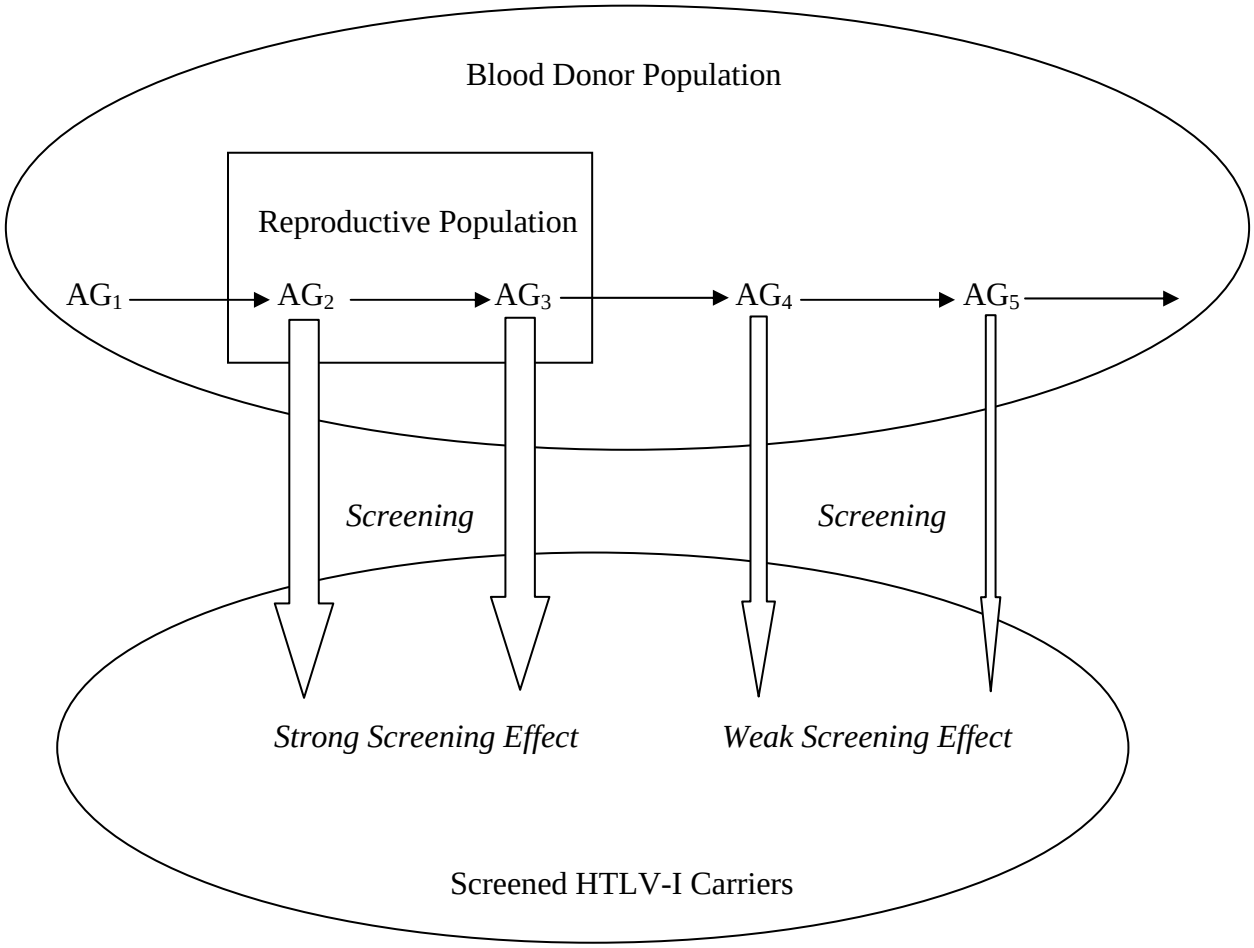

Figure 1. Screening Processes of HTLV-I Carriers. The arrow "→" implies aging, and the arrow "⟹" the screening effect.

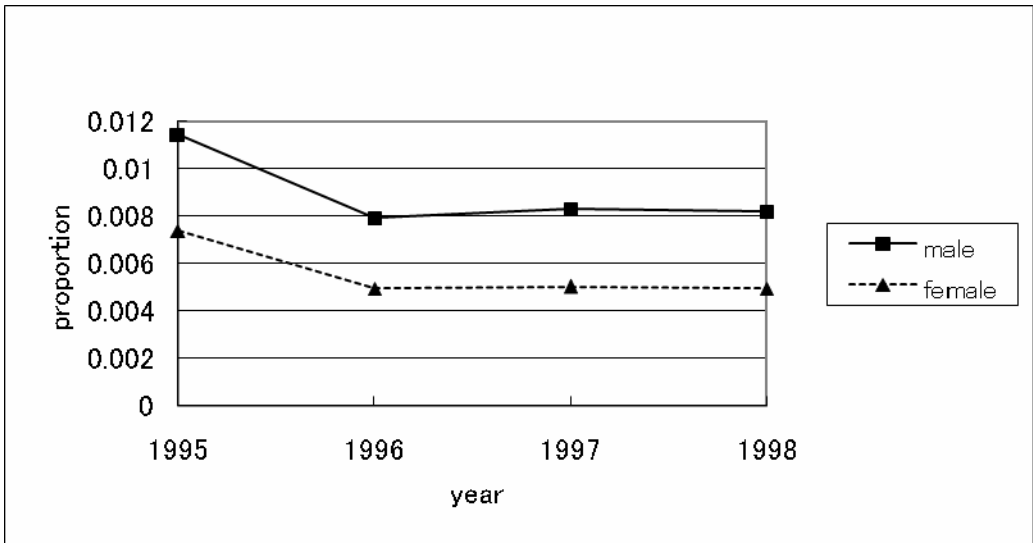

Figure 2. Changes of Carrier Proportions of Males and Females in AG_1

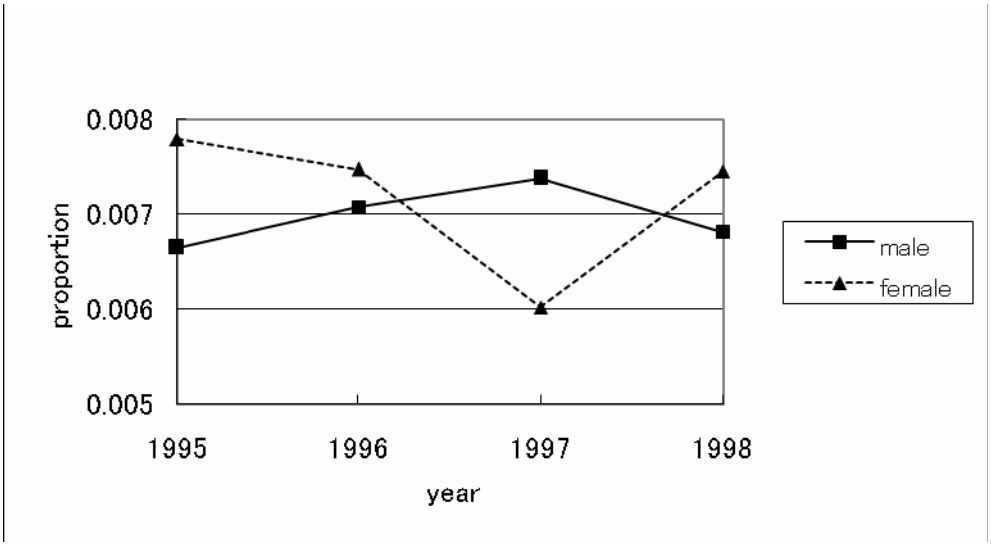

Figure 3. Changes of Carrier Proportions of Males and Females in AG_2

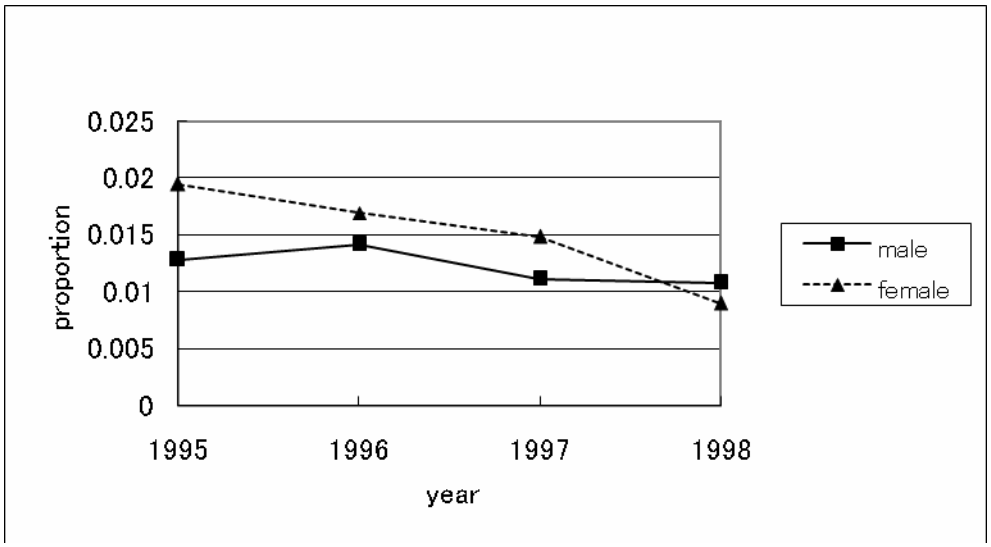

Figure 4. Changes of Carrier Proportions of Males and Females in AG_3

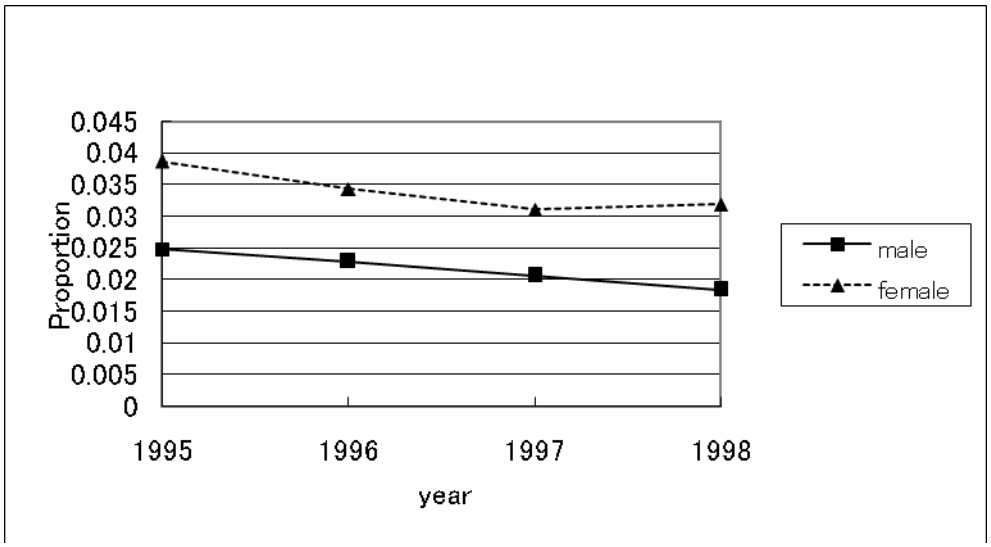

Figure 5. Changes of Carrier Proportions of Males and Females in AG_4

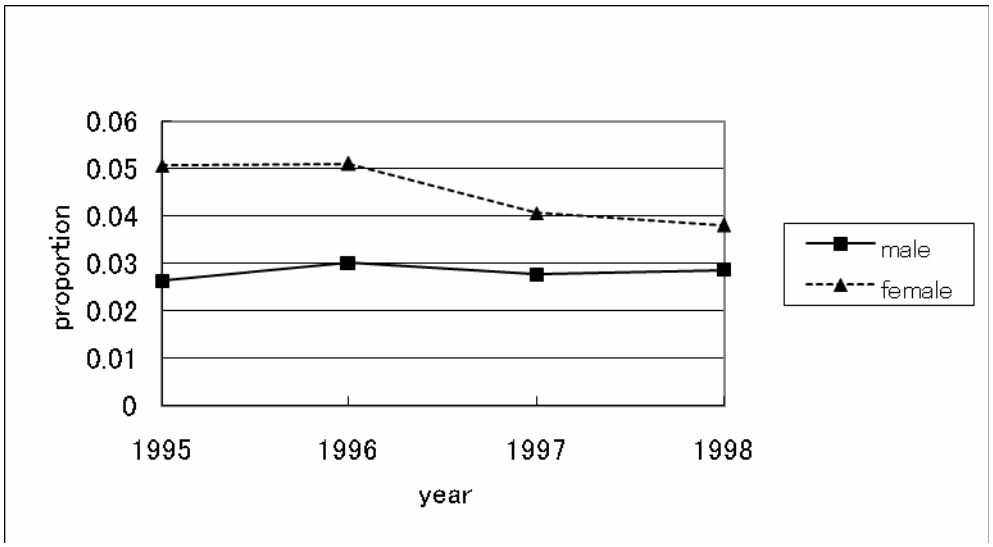

Figure 6. Changes of Carrier Proportions of Males and Females in AG_5